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Changing trends in mastitis

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ABSTRACT

The global dairy industry, the predominant pathogens causing mastitis, our understanding of mastitis pathogens and the host response to intramammary infection are changing rapidly. This paper aims to discuss changes in each of these aspects. Globalisation, energy demands, human population growth and climate change all affect the dairy industry. In many western countries, control programs for contagious mastitis have been in place for decades, resulting in a decrease in occurrence of *Streptococcus agalactiae* and *Staphylococcus aureus* mastitis and an increase in the relative impact of *Streptococcus uberis* and *Escherichia coli* mastitis. In some countries, *Klebsiella* spp. or *Streptococcus dysgalactiae* are appearing as important causes of mastitis. Differences between countries in legislation, veterinary and laboratory services and farmers' management practices affect the distribution and impact of mastitis pathogens. For pathogens that have traditionally been categorised as contagious, strain adaptation to human and bovine hosts has been recognised. For pathogens that are often categorised as environmental, strains causing transient and chronic infections are distinguished. The genetic basis underlying host adaptation and mechanisms of infection is being unravelled. Genomic information on pathogens and their hosts and improved knowledge of the host's innate and acquired immune responses to intramammary infections provide opportunities to expand our understanding of bovine mastitis. These developments will undoubtedly contribute to novel approaches to mastitis diagnostics and control.

KEYWORDS: bovine; genetics; host; mastitis; molecular typing; pathogen

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TRENDS IN THE INDUSTRY

The dairy industry worldwide has achieved many significant advances over the last fifty years, with knowledge-based genetic selection and optimised nutrition playing important roles in the delivery of increased milk yields of improved quality in most breeds (Shook 2006). Increasingly, the health and welfare of dairy cattle is seen as vital to maximise profitability and to enhance public perception of modern dairy systems (Kelton 2006; Mulligan *et al.* 2006). The next few decades are likely to present complex challenges for dairy farmers. Many of these challenges will result from national or international policies and from commercial decisions made by leaders in different countries around the world.

The world population is anticipated to reach nine billion people by 2050. The highest rate of growth is likely to be in developing countries in Africa and Asia, while the population of continents such as Europe should remain relatively stable (UN 2004). More food than that which is currently being produced will be required to feed the world population in the future. The rate of growth in demand for agricultural products has declined from 2.2% per year over the last 30 years to an anticipated 1.5% per year until 2030 (FAO 2002). Growth is largely comprised of a higher demand for meat and milk products by increasingly

wealthy countries such as India and China (Beghin 2006). The increased demand for products derived from livestock is not however restricted to richer countries, as in some of the poorest communities in the world, production of crops or vegetables are unsustainable, resulting in heavy reliance on milk and meat (Ahmed *et al.* 2002). In many of these countries there is an increasing awareness of the benefits to humans of consumption of even very small amounts of protein, especially by the young (Bhargava 2000) and the immunocompromised (Chevalier *et al.* 1998).

The world is now a global marketplace with many primary commodities and processed products travelling thousands of miles from production to consumption. The incentives for production are evolving with the decoupling of EU subsidies for meat production, the changes in handling of milk quota within the EU, and the ongoing negotiations on world trade under the General Agreements on Tariffs and Trade (www.wto.org). The UK was 59.3% self-sufficient in food production in 2008, in comparison to 70.6% between 1995 and 1997 (DEFRA 2008). While this trend towards reduced self-sufficiency was seen as acceptable by some government departments, more recent debate has suggested that the UK public may be increasingly interested in issues of food security and supply. The last

few years have seen a decline in the milk price in the UK. In the spring of 2007, prices began to increase and a price of 25.62 pence per litre was reported in February 2008. The reasons for this relatively sudden increase in price probably included flooding in the UK, concern about the reliability of supply from major retailers and an increase in global demand for liquid milk and dairy products (www.defra.gov.uk).

Current concerns about global warming and the adverse effect of greenhouse gases have influenced the perception of livestock farming, including the dairy industry. Approximately 10% of the total EU greenhouse gas emission has been attributed to agriculture (Lovett *et al.* 2006). Dairy cattle have also been associated with poor water quality arising from nitrate and phosphate excretion in some heavily-grazed areas. Opinions expressed on the competition between the use of grain as foodstuffs for humans or cattle, or for biofuels are intense and varied, and are at least in part driven by a 60% increase in cereal prices since 2005 (Müller *et al.* 2008). The significant demand for grain for whatever purpose adds pressure to an already severely pressurised dairy industry. This is likely to lead to further reductions in farm numbers with a resulting increase in the average herd size in surviving units. Climate change has been predicted to affect human morbidity and mortality significantly (Patz *et al.* 2005) and is likely to reduce the area of landmass available for agricultural purposes due to drought or flooding in many regions of the world. The Intergovernmental Panel on Climate Change (IPCC) has published detailed assessments of the effects, possible causes and options for mitigation of climate change (www.ipcc.ch). Yet another possible factor potentially adversely affecting production and trade of milk and milk products is the risk of agroterrorism aimed at the contamination or adulteration of food producing animals or their products (Wein and Liu 2005; Buttars *et al.* 2006).

In Europe and North America, current market demand and price for organic milk encourage dairy producers to consider organic production to improve the economic viability of their farm operations (Rotz *et al.* 2007). Milk from herds managed organically or conventionally, with or without use of recombinant bovine somatotropin (rbST), is similar in composition (Ellis *et al.* 2006, 2007; Vicini *et al.* 2008). There are, however, perceived nutritional and taste benefits among consumers of organic milk (Hill and Lynchehaun 2002). Environmental and health benefits are also cited as reasons for consumption of organic milk (Hill and Lynchehaun 2002), and consumers are willing to pay a high price for organic or rbST-free milk (American Farm Bureau 2008). On the other hand, environmental benefits as well as animal health benefits have been attributed to use of rbST in milk production (Allore and Erb 2000; Capper *et al.* 2008). Animal health did not appear to differ between organic and non-organic farms in the UK (Langford *et al.* 2009). Udder health was better in organic

than conventional herds in Sweden (Hamilton *et al.* 2006), whereas poor udder health caused more culling in organic than conventional herds in Switzerland (Roesch *et al.* 2006). Comparison of disease rates between conventional and organic herds can be difficult because perception and detection of disease is influenced by management system (Ruegg 2008). Despite the media attention devoted to organic dairy products and large relative increases in demand for organic dairy products, consumption of organic products is mostly limited to affluent consumers, and organic consumption constitutes a small segment of total dairy consumption (Hill and Lynchehaun 2002).

The dilemma, which the dairy industry will have to face, is how to maximise production without compromising welfare, while reducing the impact of carbon emissions from dairy cattle. An important aspect of achieving these aims is to focus on overall lifetime productivity of cattle, rather than on traditional annual targets (VanRaden 2004). To achieve this aim, a reduction in morbidity and mortality due to preventable and prevalent endemic diseases is needed, one such disease being mastitis. If this is to become a reality, increased efforts are required in the speed and accuracy of detection of mastitis, better targeting of therapy, and improved management and decision making in prevention of exposure to pathogens and avoidance of significant risk factors for disease (LeBlanc *et al.* 2006). If successful, a holistic approach to preventing and controlling mastitis may benefit communities across the world, in both nutritional and environmental terms.

TRENDS AT PATHOGEN LEVEL

In most countries, the major mastitis pathogens are *Streptococcus agalactiae*, *Streptococcus dysgalactiae* and *Streptococcus uberis*, *Staphylococcus aureus*, and *Escherichia coli*. The word "major" reflects their considerable impact on cow health, milk quality and productivity. When the standard mastitis prevention programme was developed in England (Neave *et al.* 1969), Gram-positive pathogens such as *Streptococcus* and *Staphylococcus* species were the predominant causative agents of mastitis. Many infections caused by these pathogens were subclinical and chronic. The standard mastitis prevention programme aimed to reduce the prevalence of such infections by limiting the number of new infections and the duration of infections, recognizing that incidence and duration are the driving forces behind prevalence (Dodd *et al.* 1969). Implementation of these measures lead to a reduction in prevalence of *Strep. agalactiae*, *Strep. dysgalactiae*, *Staph. aureus*, and, to a lesser extent, *Strep. uberis* in individual herds (Neave *et al.* 1969). As more herds implement the standard control measures, pathogen populations at regional or national level start to shift. In some European countries, e.g. Belgium, Denmark, Norway and the UK, occurrence of *Strep. agalactiae* is now sporadic (Andersen *et al.* 2003; Østerås *et al.* 2006; Bradley *et al.* 2007; Piepers *et al.* 2007). In other areas, *Strep. agalactiae* is still quite

common, e.g. in parts of Europe [Germany: 29% of herds positive (Tenhagen *et al.* 2006)] and the Americas [Brazil: 60% of herds positive (Duarte *et al.* 2004); Uruguay: 11% of herds positive (Giannechini *et al.* 2002); New York, USA: 10% of cows positive (Wilson *et al.* 1997)]. Subclinical mastitis constitutes a major source of economic loss for dairy farms due to unrealised production potential and decreased milk quality (Hortet and Seegers, 1998; Huijps *et al.* 2008). The feasibility (Neave *et al.* 1969) and economic benefit (Yamagata *et al.* 1987; Edmondson 1989; Erskine and Eberhart 1990) of *Strep. agalactiae* control have been demonstrated repeatedly. This should provide every producer with the incentive to control *Strep. agalactiae* mastitis, and to use resources for milk production in a more efficient manner. In reality, motivation of producers to control mastitis may depend on bonus and penalty systems (Dekkers *et al.* 1996; Allore and Erb 1998; Nightingale *et al.* 2008). Regulatory limits for bulk milk somatic cell counts (BMSCC), which reflect mastitis prevalence in a herd (Eberhart *et al.* 1982), differ between countries or are entirely lacking. In Colombia, for example, the dairy industry cannot keep up with current demand. The need for milk quantity outweighs the need for milk quality, and there is currently no legislation or laboratory capacity to regulate and measure BMSCC in Colombia. Producers' ability to control mastitis will depend on access to knowledge, diagnostic facilities, and animal health products. An increasing number of cows are milked in Latin America or by Hispanic milkers in the USA. To provide Spanish-speakers with access to information on mastitis control, a growing number of organisations provide bi-lingual milker training and translation of educational materials (NMC 2008; Quality Milk Production Services 2008; University of Wisconsin 2008).

In parallel with the decreases in national average BMSCC and prevalence of *Strep. agalactiae*, a decrease in prevalence of *Staph. aureus* has been seen in some countries. In Finland, BMSCC decreased from 320,000 cells/ml in 1990 to 180,000 cells/ml in 1995 and *Staph. aureus* prevalence decreased from 31% to 17% of isolations (Myllys *et al.* 1998). As BMSCC and prevalence of major pathogens decrease, the relative importance of other organisms, particularly coagulase negative staphylococci (CNS), increases. In the Netherlands, the prevalence of CNS among bacterial isolates from milk samples increased from 16.2% in 1999 to 42.2% in 2004 for subclinical mastitis, and from 7.3% to 14.1% for clinical mastitis (Sampimon *et al.* 2007). CNS are now among the most commonly isolated organisms from milk samples of cows with subclinical mastitis in many countries (Rajala-Schultz *et al.* 2004; Østerås *et al.* 2006; Tenhagen *et al.* 2006; Bradley *et al.* 2007). In 2007, the first international symposium on CNS in bovine mastitis was organised, reflecting the increasing recognition of the potential importance of this group of pathogens (De Vliegher *et al.* 2009). Molecular methods for identification of CNS provide accurate tools for epidemiological studies at CNS

species level and future development of species-specific recommendations for treatment and prevention of CNS mastitis (Zadoks and Watts 2009).

Despite improvements in control of subclinical mastitis, control of clinical mastitis continues to constitute a major challenge (Hillerton *et al.* 1995). The main organisms associated with clinical mastitis in England and Wales are *E. coli* and *Strep. uberis* (Milne *et al.* 2002; Peeler *et al.* 2003; Bradley *et al.* 2007). A steady increase in relative importance of *Strep. uberis* can be seen over the decades, with 15, 17, 21 and 24.5% of clinical mastitis cases attributed to this species in 1969, 1980, 1993 and 2005, respectively (Neave *et al.* 1969; Bramley 1984; Hillerton *et al.* 1993; Bradley *et al.* 2007). When accounting for the incidence of clinical mastitis per 100 cows per year, however, the absolute incidence of *Strep. uberis* mastitis decreased from 21 cases per 100 cows in 1969 (Neave *et al.* 1969), to only seven or eight cases per 100 cows in 1980 (Bramley 1984) and 1993 (Hillerton *et al.* 1993). In recent years, reported *Strep. uberis* incidence has increased again, with 16 cases/100 cows/year reported for 2005 (Bradley *et al.* 2007). The second most common group of pathogens causing clinical mastitis in England and Wales consists of *E. coli* and other Enterobacteriaceae (Milne *et al.* 2002; Bradley *et al.* 2007). In New Zealand, as in the UK, *Strep. uberis* is the most common pathogen associated with clinical mastitis, but in contrast to the situation in the UK, *E. coli* mastitis is rarely observed (McDougall 2003). In Norway, neither *Strep. uberis* nor *E. coli* are commonly found. Rather, *Staph. aureus* and *Strep. dysgalactiae* are the most common causes of clinical mastitis (Whist *et al.* 2007). *Staphylococcus aureus* is the main pathogen associated with clinical mastitis in The Netherlands, followed by *E. coli* and *Strep. dysgalactiae* (Barkema *et al.* 1998). *Mycoplasma* (González and Wilson 2003; Fox *et al.* 2005) and *Klebsiella* (Hoe and Ruegg 2005; Munoz *et al.* 2007; Paulin-Curlee *et al.* 2007) mastitis are major concerns in the USA. Faecal shedding of *Klebsiella* is thought to contribute to exposure and mastitis incidence in dairy herds in the USA (Munoz *et al.* 2006; 2007). By contrast, *Klebsiella* mastitis outbreaks are only occasionally reported from Europe and they are predominantly associated with contaminated sawdust bedding (Vecht *et al.* 1987; Sampimon *et al.* 2006). *Mycoplasma* mastitis is only occasionally reported from Europe (Blackburn *et al.* 2007; Tolboom *et al.* 2008). Increasingly, milk samples from clinical mastitis cases are culture-negative. Culture-negative samples were obtained from approximately 25 to 30% of clinical cases in studies in the UK (Milne *et al.* 2002; Bradley *et al.* 2007) and the USA (Hoe and Ruegg 2005). It has been proposed that culture negative cases should be attributed to coliform organisms, and that our diagnostic methods and criteria, which were developed for detection of chronic, subclinical infections, need to be updated to be appropriate for the changed spectrum of mastitis pathogens (Bradley *et al.* 2005). Use of PCR based methods may also contribute

to improved mastitis diagnostics, e.g., through increased sensitivity of detection of *Staph. aureus* (Graber *et al.* 2007) or organisms that are non-viable or difficult to culture (Barlow *et al.* 2008).

Considering that different pathogens are the predominant cause of clinical mastitis in different countries, mastitis controls will need to be developed to meet the specific requirements of an individual country or segment of the dairy industry. Some mastitis control strategies, e.g., vaccination against coliform mastitis, are widely adopted in one country (USA) but may not be available in other countries (e.g. The Netherlands) and not even relevant elsewhere (New Zealand, Norway). Targeted intervention strategies are being developed and implemented in a number of countries (Green *et al.* 2007; Lam *et al.* 2007; www.countdown.org.au). Significant improvements in udder health have been reported (Green *et al.* 2007), but adoption and success rates depend on farmer motivation and behaviour (Lam *et al.* 2007). Insight into socio-economic factors that motivate farmers to control mastitis may be as important as scientific or technical knowledge to improve uptake of mastitis control measures (Lam *et al.* 2007).

TRENDS AT STRAIN LEVEL

The application of molecular or DNA-based methods in mastitis research and diagnostics has contributed to an increased understanding of mastitis epidemiology and control options. Using these methods, isolates belonging to a bacterial species can be differentiated at the subspecies or strain level, allowing for improved recognition of sources and transmission routes of pathogens (Zadoks and Schukken 2006).

Streptococcus agalactiae is the quintessential contagious mastitis pathogen. It mostly causes subclinical infections and implementation of the standard mastitis prevention programme has all but eradicated this organism from several countries in Western Europe. Occasionally, clinical *Strep. agalactiae* mastitis is observed in herds with good control of contagious mastitis, as evidenced by low BMSCC (< 150,000 cells/ml) (Barkema *et al.* 1998). Clinical episodes of *Strep. agalactiae* mastitis can also be induced experimentally (Jensen 1982). They are generally short-lived and do not result in transmission of the pathogen in the herd. Occurrence of *Strep. agalactiae* in bulk tank milk from herds without evidence for transmission or presence of the pathogen in individual cow has been attributed to cross contamination between loads of milk (Andersen *et al.* 2003). Other authors attribute the occurrence of incidental clinical mastitis and the presence of *Strep. agalactiae* in bulk tank milk obtained from *Strep. agalactiae*-free animals to introduction of human *Strep. agalactiae* strains (Zadoks and Schukken 2006). *Strep. agalactiae* or Group B *Streptococcus* (GBS) is highly prevalent in humans, with carriage rates of 30% or more in the urogenital and distal gastrointestinal tracts of

women (Manning *et al.* 2000; Meyn *et al.* 2002; Hansen *et al.* 2004) and close to 25% in men (Manning *et al.* 2000). Maternal carriage of *Strep. agalactiae* may result in neonatal infection, with subsequent sepsis, meningitis, permanent neurological damage, or death of newborns. Human and bovine isolates largely constitute genetically distinct subpopulations within the bacterial species (Bisharat *et al.* 2004; Bohnsack *et al.* 2004; Dogan *et al.* 2005; Sukhnanand *et al.* 2005). Canine and feline strains of *Strep. agalactiae* are genetically similar to human rather than bovine strains (Yildirim *et al.* 2002). A virulent human strain of *Strep. agalactiae* that is significantly associated with invasive neonatal disease has evolved from bovine strains of *Strep. agalactiae* (Bisharat *et al.* 2004). To prevent emergence of additional human-pathogenic strains from a bovine *Strep. agalactiae* reservoir, it has been suggested that the pathogen should be eradicated from the cattle population (Hillerton *et al.* 2004). *Strep. agalactiae* is highly prevalent in humans but relatively rare in UK dairy herds, and its presence in dairy herds may be the result or the cause of its presence in humans. Thus, it is uncertain whether elimination from cattle would be a cost-effective way to prevent human disease due to *Strep. agalactiae*.

Staphylococcus aureus has been more difficult to control using the standard mastitis prevention programme than *Strep. agalactiae*. This is due in part to difficulty in diagnosing and curing *Staph. aureus* infections (Barkema *et al.* 2006). In addition, *Staph. aureus* can be found in many sources, including bovine skin, non-bovine animals and humans, flies, and the barn environment. Strain typing studies show that some cases of *Staph. aureus* mastitis are not due to contagious transmission but environmental in origin (Kapur *et al.* 1995; Rivas *et al.* 1997; Zadoks *et al.* 2000; Joo *et al.* 2001). The incidence of environmental *Staph. aureus* infections would not be affected by implementation of the standard mastitis prevention programme. Across countries and continents, more than 85% of bovine *Staph. aureus* mastitis is caused by a genetically closely related subpopulation of the species (Fitzgerald *et al.* 1997; Smith *et al.* 2005), which is adapted to the bovine host or, more specifically, to the bovine udder (Herron-Olson *et al.* 2007). Strains of *Staph. aureus* differ in their ability to spread from cow to cow (Smith *et al.* 1998; Zadoks *et al.* 2002) or to provoke an increase in somatic cell count (Zadoks *et al.* 2002), in impact on milk yield (Middleton and Fox 2002), and in response to antimicrobial treatment (Barkema *et al.* 2006). The full genome sequences of a number of *Staph. aureus* strains are now available (Baba *et al.* 2008). This allows for detailed examination of genetic elements or proteins that may be responsible for host or tissue adaptation (Herron-Olson *et al.* 2007). An improved understanding of such components may contribute to the development of new intervention strategies to control *Staph. aureus* mastitis (Fitzgerald *et al.* 2000; Mullarky *et al.* 2001). As for *Strep. agalactiae*, a virulent strain

that poses a potential human health risk has arisen from a subpopulation of bovine *Staph. aureus* (Guinane *et al.* 2008). In addition, methicillin-resistant *Staph. aureus* in dairy cattle and cattle farmers is an increasing concern (Juhász-Kaszanyitzky *et al.* 2007; Moon *et al.* 2007; Van Loo *et al.* 2007). These findings lend new urgency to the need to improve control of *Staph. aureus* mastitis.

Whilst *Staph. aureus* is a contagious pathogen more than an environmental pathogen, the reverse is true for *Strep. uberis* (Zadoks 2007). Strain typing studies have revealed large genetic heterogeneity among *Strep. uberis* isolates obtained from animals within a single herd, particularly in New Zealand (Douglas *et al.* 2000; Wieliczko *et al.* 2002; McDougall *et al.* 2004; Pullinger *et al.* 2006), which proves absence of cow-to-cow transmission. In other studies, however, predominant strains of *Strep. uberis* have been observed within herds (Zadoks *et al.* 2003; Coffey *et al.* 2006), which might be explained by contagious transmission. In one outbreak, the milking machine was identified as a likely fomite for such transmission (Zadoks *et al.* 2003). Alternative explanations for strain predominance include overrepresentation of a strain in the environment, or host-adaptation of a subset of the *Strep. uberis* population (Zadoks and Schukken 2006). In studies from New Zealand, several common environmental strains were underrepresented among mastitis causing isolates (Lopez-Benavides *et al.* 2007; Pullinger *et al.* 2006), which argues against environmental strain predominance as a full explanation for strain predominance among bovine isolates. Modern genomic and proteomic methods enable us to explore the host-adaptation hypothesis (Zadoks 2007) as well as potential vaccine targets (Leigh *et al.* 2004). They may also help us understand why some *Strep. uberis* infections respond well to antimicrobial treatment, whereas others do not respond to prolonged intramammary therapy (Milne *et al.* 2005). In vitro, adherence of *Strep. uberis* and subsequent invasion and survival in mammary epithelial cells has been observed (Tamilselvam *et al.* 2006). Such processes could explain persistence of infection and treatment failures, but it is uncertain whether these observations are a reflection of events that take place in vivo (Leigh 1999). Use of infection models in animals will help to assess the relevance of these experiments and their potential contribution to our knowledge of disease causation and control.

Where *Strep. agalactiae* may be considered the quintessential contagious pathogen, *E. coli* can be considered the quintessential environmental pathogen and no evidence for cow-to-cow transmission of *E. coli* has been published yet. There is, however, emerging evidence for host-adaptation of *E. coli*. Most *E. coli* infections occur in early lactation in high producing cows, when immunosuppression through metabolic deficiencies (Suriyasathaporn *et al.* 2000a) or extremely low SCC (Suriyasathaporn *et al.* 2000b; Peeler *et al.* 2003) lowers

the host's resistance. Infections tend to result in acute and often severe clinical mastitis with removal of the pathogen or removal of the cow as the two most likely outcomes. In other animals, however, recurrent episodes of clinical mastitis are observed, with considerable economic loss as a result (Bar *et al.* 2007). Recurrent clinical episodes may be due to cure and reinfection of a susceptible host. Alternatively, the bacteria and the cow may survive simultaneously, and subclinical episodes of a persistent infection may alternate with clinical episodes. Strain typing studies have confirmed the existence of both mechanisms (Döpfer *et al.* 1999, 2000; Bradley and Green 2001; Dogan *et al.* 2006). Persistence of *E. coli* infections is associated with invasion and survival of *E. coli* inside mammary epithelial cells in vitro (Dogan *et al.* 2006), a mechanism similar to that proposed for *Strep. uberis*. Virulence genes typically associated with invasion of diarrhoeagenic and uropathogenic pathogenic *E. coli* did not account for the invasive behaviour of mastitis-associated *E. coli* strains and the mechanism of invasion is as yet undetermined (Dogan *et al.* 2006). Whole genome comparison of *E. coli* strains associated with different disease syndromes may reveal genes that are specific to mastitis associated *E. coli*. Some cases of clinical *E. coli* mastitis that are observed in lactation are the result of infections that took place in the dry period (Bradley and Green 2000), implying that the dry period rather than peak lactation can be a critical time for control of *E. coli* mastitis. Use of internal teat sealants does not reduce the risk of dry-period infections with coliform bacteria significantly (Berry and Hillerton 2002; 2007)

Through use of genomic and proteomic tools, it will be possible to further explore how different subpopulations of pathogens differ in mechanisms of disease causation and transmission. A more developed understanding of the pathogenesis and epidemiology of bovine mastitis will provide new approaches aimed at improvements in mastitis control. Rapid, effective, and increasingly cost-effective methods of sequencing pathogen genomes have already contributed new information to our understanding of host-pathogen interactions in mastitis. Manipulation of mastitis pathogens using molecular methods such as mutagenesis (Brouillette and Malouin 2005), and measurement of messenger RNA (transcriptomics), proteins or peptides (proteomics) and networks of metabolites (metabolomics), provide further opportunities to investigate mastitis pathogenesis at pathogen and host level (Lippolis and Reinhardt 2005; Smolenski *et al.* 2007; Taverna *et al.* 2007; Boehmer *et al.* 2008).

TRENDS AT HOST LEVEL

In many countries, the emphasis on improving dairy production has been to focus on breeds that have particularly high milk yields, especially Holstein, which is the most widespread dairy cattle breed, found in 128 countries worldwide (FAO 2007). In the USA, fewer than 5% of dairy cattle belong to breeds other than purebred

or grade Holsteins (McAllister 2002). There has been a decrease in the numbers of more traditional breeds such as Jersey and Guernsey and Alpine breeds, which have lower milk yields but better quality milk than the Holstein breed in terms of benefits to human health or processing qualities (Ellis *et al.* 2006; De Marchi *et al.* 2007; Kamiski *et al.* 2007). Selection of dairy cattle over the years has been based on phenotypic traits such as teat length, udder placement and 'dairy type', however, increased emphasis on selection of sires for traits such as predicated transmitting ability for somatic cell counts, an indirect measurement of mastitis susceptibility, and for economic traits such as profit index, total economic index and profitable life index, are becoming commonplace (Dekkers and Gibson 1998; Wicks and Leaver 2006). Selection of sires for these traits has increased the rate of change of phenotypic characteristics, for example milk yield and quality, however, this has occurred through selection of a relatively small number of very popular sires, especially within Holstein Friesians. In some animal species, selection of a small number of sires has been associated with genetic bottlenecks (Mikko and Andersson 1995; Rendo *et al.* 2004). This may result in reduced variation in the offspring, which may in turn have adverse effects on health and welfare. One gene complex which has been long recognised as being associated with mastitis susceptibility or resistance is the Major Histocompatibility Complex, where Class I (Vage *et al.* 1992) were found to be associated with health traits including measurements of somatic cell counts (Weigel *et al.* 1990) and Class II (Sharif *et al.* 2003) genes have been demonstrated as playing an important role in presenting antigen to cells of the immune system and in response of lymphocytes to mastitis pathogens (Park *et al.* 1993). More recently, other genes and gene complexes have been shown to play a role in the pathogenesis of mastitis, including Toll-like receptors (Opsal *et al.* 2008).

In some countries, priorities such as the adaptability of cows to once a day milking or for specific systems including low input or extensive management, have led to selection for characteristics other than milk yield. There is increasing interest in cross-breeding dairy cattle such as Norwegian Reds with one or more breeds (e.g. Holstein Friesian and/or Normande, Montbeliarde; Heins *et al.* 2006) in order to produce dairy cattle with reasonable production levels combined with a reduced susceptibility to some of the main production diseases including mastitis and endometritis (Heringstad *et al.* 2007). Changing breeding goals also reflect a growing public interest in sustainability of dairy cattle production, with consideration of market economic values and non-market values such as animal welfare (Oltenacu and Algers 2005; Nielsen *et al.* 2006). In contrast to the dominance of the Holstein breed in terms of cow numbers and contribution to dairy genetics, certain dairy breeds across the world have had their numbers reduced to such an extent, that their sustainability is threatened. One such example is the

Modicana breed of cattle, originating in Sicily where they produce very high quality hard cheese: Ragusano (Licitra *et al.* 1998). It is important that a genetic resource for minor breeds is maintained in order to protect against extinction and to ensure that beneficial genes could be re-introduced to the dairy cattle population should they be identified as required. The uncertainty of the direction of the dairy industry in terms of supply and demand, and in light of potential environmental impacts, makes maintenance of diversity in dairy cattle particularly important.

Requirements for liquid milk and dairy products in developing countries will require development of more efficient dairying systems and investment in the application of modern technologies where appropriate. Maintenance of dairy cattle in developing countries poses many challenges, especially breeding cattle, which are resistant to many of the diseases with high mortality such as theileriosis and trypanosomiasis (Freeman *et al.* 2004). In East Africa, dairy cattle are principally cross-breeds between European *Bos taurus* dairy breeds, including Ayrshire, Friesian or Jersey, and indigenous *Bos indicus* cattle such as Boran or Zebu (Msanga *et al.* 2000; Karimuribo *et al.* 2006). The Boran breed which is recognised for its relatively high milk yield compared to other cross-breeds, has been shown to be 3.4 times more likely to be affected by clinical and subclinical mastitis than the Tanzanian Shorthorn Zebu breed (Karimuribo *et al.* 2006). Selection of crossbred dairy cattle on the basis of greater milk yield may therefore need to be combined with an improvement in control programmes for mastitis to ensure a sustainable industry.

Resistance to mastitis has been associated with both innate (Rainard and Riollet 2006) and acquired immunity (Kehrli and Harp 2001). Selection of cattle for breeding for mastitis resistance depends on measurement of humoral or cellular assays which are predicted as correlates of protection against intramammary infection (Dosogne *et al.* 2002; Shkreta *et al.* 2004). It is increasingly recognised that different mastitis species induce very different inflammatory and immune responses. Examples include the very rapid and relatively short-lived influx of neutrophils following infection with the Gram negative organisms such as *E. coli* (Mehrzhad *et al.* 2004) and the more gradual concentration of lymphocytes, including regulatory T cells in mammary glands infected with *Staph. aureus* (Chang *et al.* 2005). Host genetics affect disease incidence and severity, and pathogen genetics may influence the host genetic structure. Therefore, the evolutionary impact of the interaction between the host and its pathogens needs to be taken into consideration to fully appreciate implications of breeding strategies aimed at mastitis prevention (Detilleux 2009).

Host responses to intramammary infection differ between pathogen species (Bannerman 2008; Bannerman *et al.* 2004a, b) and pathogen strains. The ability of the host

to prevent establishment of new intramammary infections is determined in part by the ability of neutrophils to phagocytose and kill pathogens. Pathogen strains that evade neutrophil killing are more likely to establish infection than those that do not. In vitro, *Staph. aureus* strains that produce enterotoxins are not killed as efficiently as enterotoxin negative strains (Mullarky *et al.* 2001). In a large collection of *Staph. aureus* isolates from three continents, enterotoxin producing strains were overrepresented among isolates that caused clinical mastitis (Mullarky *et al.* 2001), suggesting that the inability of the host immune system to deal efficiently with these strains results in increased likelihood of clinical disease. Whether it is the enterotoxins themselves that affect the immune response, or other virulence factors encoded by the *agr*-loci that enterotoxin production tends to be associated with, is not fully understood (Mullarky *et al.* 2001). For *Strep. uberis*, differences between strains in their ability to cause clinical mastitis have also been associated with differences in their ability to resist phagocytosis and killing by host neutrophils (Leigh *et al.* 1990). Analysis of large international collections of *Strep. uberis* has shown that isolates that carry the *hasA* gene, which encodes hyaluronate synthase and contributes to capsule formation, are more likely to cause clinical mastitis than other isolates (Coffey *et al.* 2006; Pullinger *et al.* 2006; Tomita *et al.* 2008). Initially, it was thought that capsule mediates resistance of *Strep. uberis* to phagocytosis and killing by bovine neutrophils (Leigh 1999), but later studies showed that the presence of capsule is not directly related to the bactericidal action of neutrophils (Field *et al.* 2003). Thus, the mechanisms underlying strain-specific host-immune responses to *Staph. aureus* and *Strep. uberis* remain to be elucidated. Mechanisms underlying strain specific interaction of pathogens with host mammary epithelial cells, as described for *Strep. uberis* (Matthews *et al.* 1994) and *E. coli* (Dogan *et al.* 2006) have not been identified either. Further studies are required to compare inter-strain variation in host response.

SUMMARY AND OUTLOOK

While this manuscript describes the trends considered by the authors as impacting significantly on the dairy and veterinary industries, many aspects discussed also potentially relate to infections of the mammae of humans and identification of the causes and effects of intramammary disease in lactating women. Breast-feeding mothers are at risk of clinical and possibly subclinical mastitis (Tomkins 2000; Foxman *et al.* 2002) and infectious mastitis is one of the main causes of premature weaning in lactating women (Delgado *et al.* 2008). The current focus on 'One Medicine' by the Centres for Disease Control and Prevention in the USA and the promotion of this approach by the editors of the British Medical Journal and The Veterinary Record (Alder and Easton 2005) aims to combine research efforts on human and veterinary diseases to provide

benefits to all mammals in terms of effective response to terrorism, diseases and natural disasters. Some of the trends appearing in mastitis in dairy cattle raise issues of comparative medicine in addition to the impact of the disease on the availability and benefits of milk to the human population.

Recent scientific developments, specifically in the areas of genomics and proteomics at host and pathogen level, provide exponentially growing opportunities for a deepening of our understanding of mastitis pathogenesis and epidemiology. Whilst it is hoped that such scientific understanding will contribute to mastitis control, social, economic and climatic trends are likely to be the main drivers behind changes in milk production systems and mastitis control. At herd level, motivation of farmers is an essential driver. At the national level, regulatory and economic incentives may be used to drive farmer motivation. At a global level, food and fuel needs and changes in human population size, wealth distribution, climate and land availability and use will be major forces in determining the future of agriculture and dairy farming. Although there are many uncertainties surrounding estimates in anticipated population, climate and land use changes, one thing is clear: more people will need to be provided with animal-based food products using fewer natural resources. Thus, there is an urgent need to increase the efficiency of milk production. Mastitis control is a step towards reaching that goal.

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